Supporting Information for:

Predominance of anaerobic, spore-forming bacteria in active extant microbial communities from ancient Siberian permafrost

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Table S1 The gradient condition for HPLC using a mobile phase consisting of methanol and 50 mM sodium acetate (pH=5.4).

Time (min)	Flow rate (mL/min)	Pump A (50 mM sodium acetate)	Pump B (methanol)
0	0.6	98%	2%
5.5	0.6	90%	10%
16	0.6	80%	20%
18	0.6	5%	95%
22	0.6	5%	95%
25	0.6	98%	2%
30	0.6	98%	2%

Table S2 Geochemical characterization of permafrost sediments

Depth, m	Temperature (^{o}C)	pH[1]	F^-	Cl^-	SO_4^{2-}	PO_4^{3-}	NO_2^-	NO_3^-	Organic acids[2]
1.4	-2	6.2	2.4±0.17	11.2±4.32	6.2±0.63	0.4±0.25	4.5±1.46	2.1±0.41	Below detection
14.8	-6	7.6	5.1±0.27	7.4±0.75	10.9±1.21	55.6±6.31	9.8±2.07	1.7±0.18	Below detection
24.8	-5	7.5	1.9±0.02	7.6±0.41	7.5±0.70	6.1±1.08	13.3±0.75	3.4±0.91	Below detection

[1]pH and anions were determined from the water extract of the sediments.

[2]Note: Small fatty acids including formate, acetate and propionate were measured. The detection limit for formate, acetate and propionate were 0.0625 ppm.

Table S3 Summary for iDNA and eDNA yield from ancient permafrost sediment.

Sample	Concentration (ng/μL)	Final volume (μL)	Weight of permafrost used (g)	DNA yield (ng/g)
1.4eDNA	0.0644	5000	10	32.2
1.4iDNA	2.92	5000	10	1460
11.8eDNA	0.0232	100	10	0.232
11.8iDNA	0.126	100	10	1.26
24.8eDNA	0.0112	50	40	0.014
24.8iDNA	0.0430	50	30	0.072
Blank 1	Below detection (<0.01)	50	None	N/A
Blank 2	Below detection (<0.01)	50	None	N/A

Figure S1 Location of the borehole (AL1-15) close to the Alazeya River within Kolyma-Indigirka Lowland in northeastern Siberia in August, 2015.

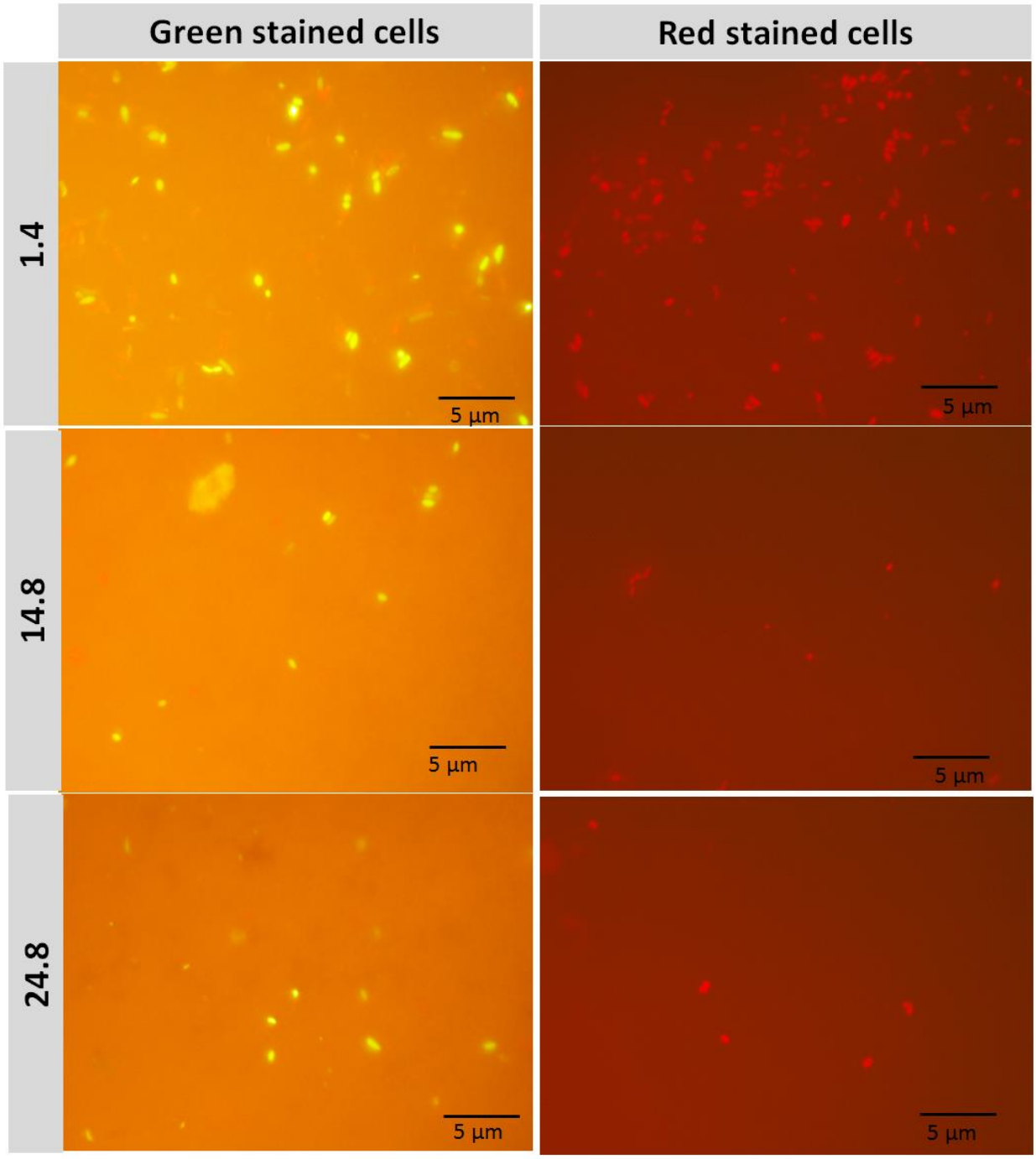

Figure S2 Live/Dead cell staining of separated cells in permafrost sediments at 1.4 (top), 11.8 (middle) and 24.8 m (bottom). The green stained live cells by Syto9 are shown in the left panel whereas the red stained cells are depicted in the right panel.

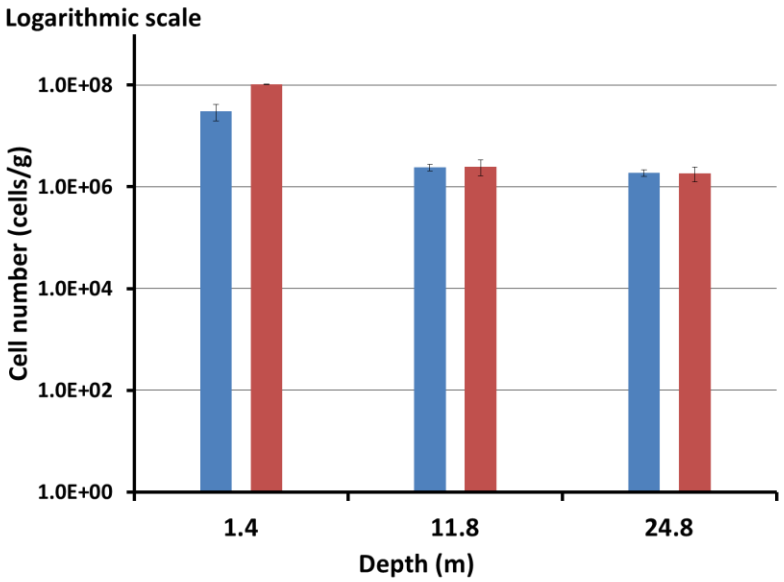

Figure S3 Cell counting of potentially live/dead cells from samples at three depths.

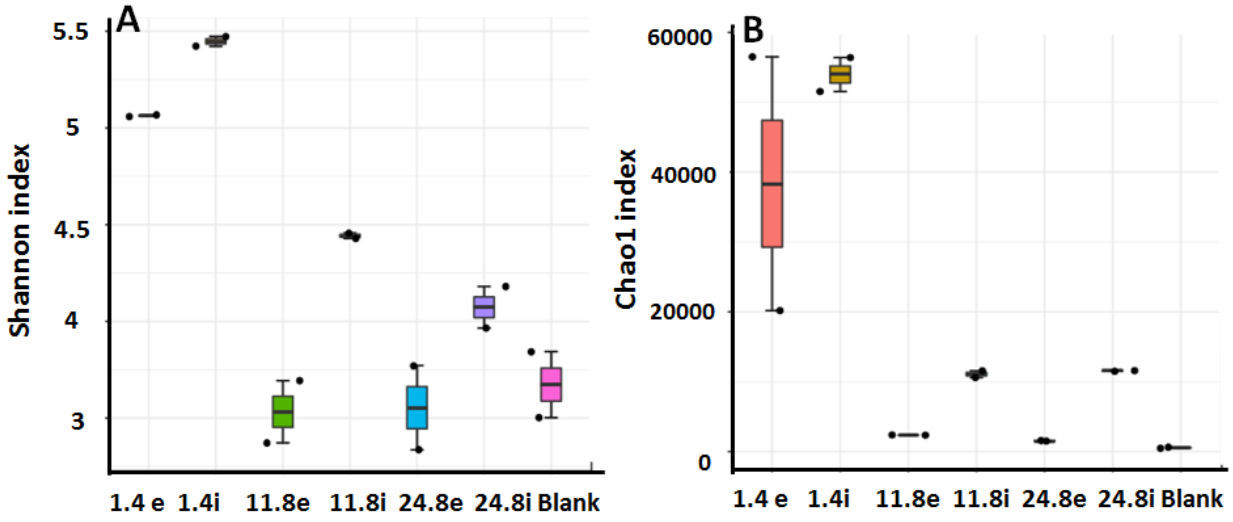

Figure S4 Comparing α-diversity of microbial communities with Shannon (A) and Chao1 (B) indices.

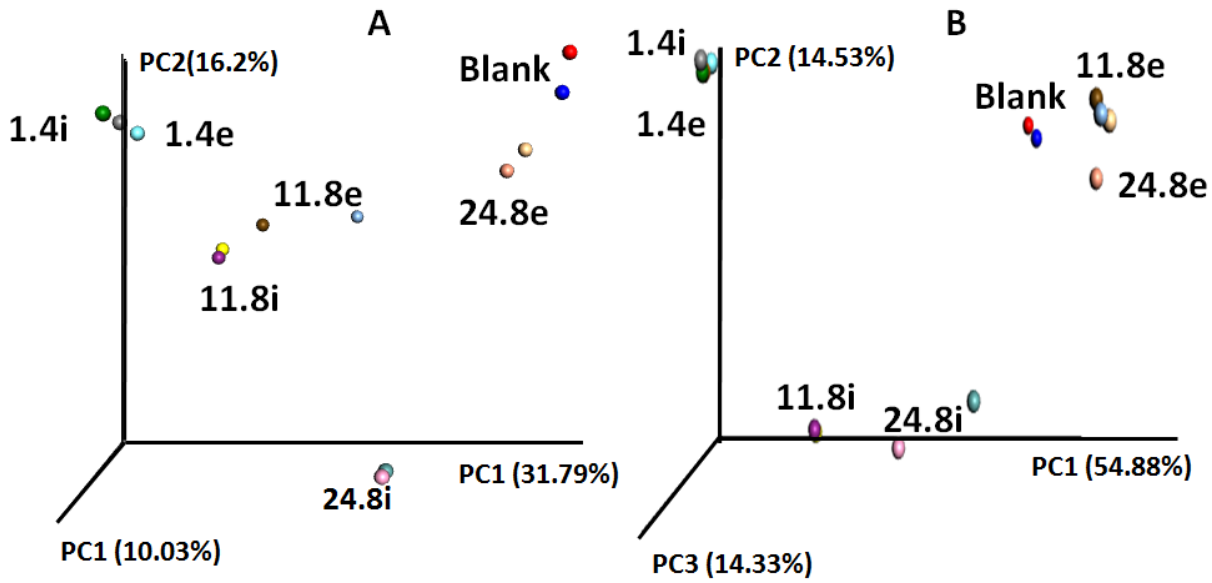

Figure S5 Principal coordinate analyses of unweighted (A) and weighted (B) UniFrac distances.

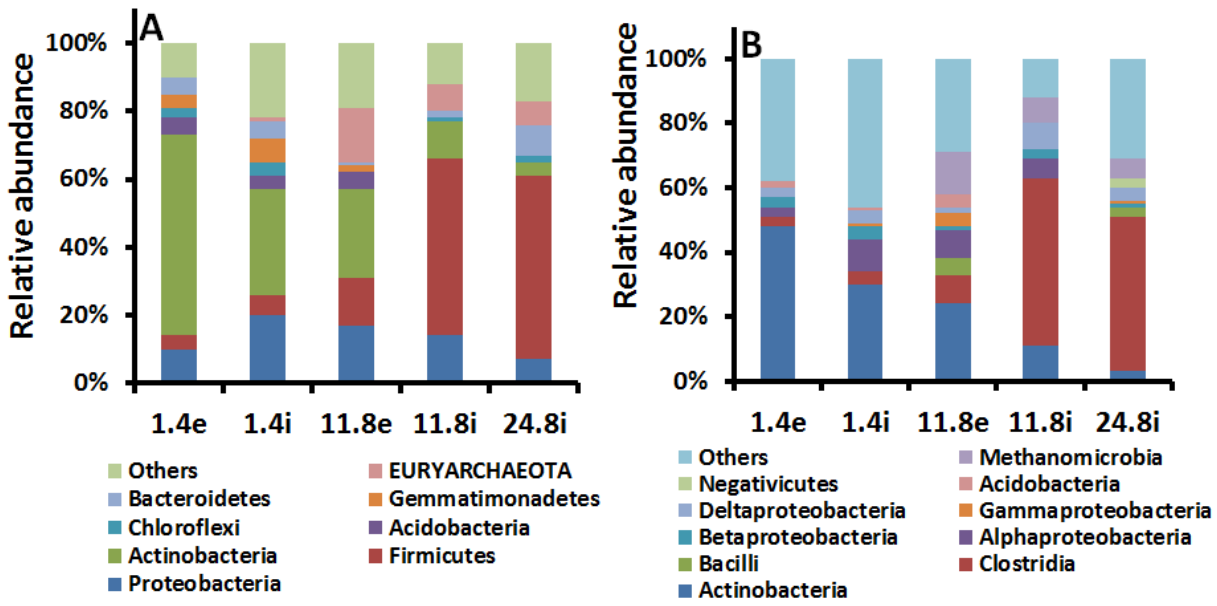

Figure S6 Microbial community profiles at phylum (A) and class level (B) inferred from metagenomes using single copy marker genes in PhyloSift.

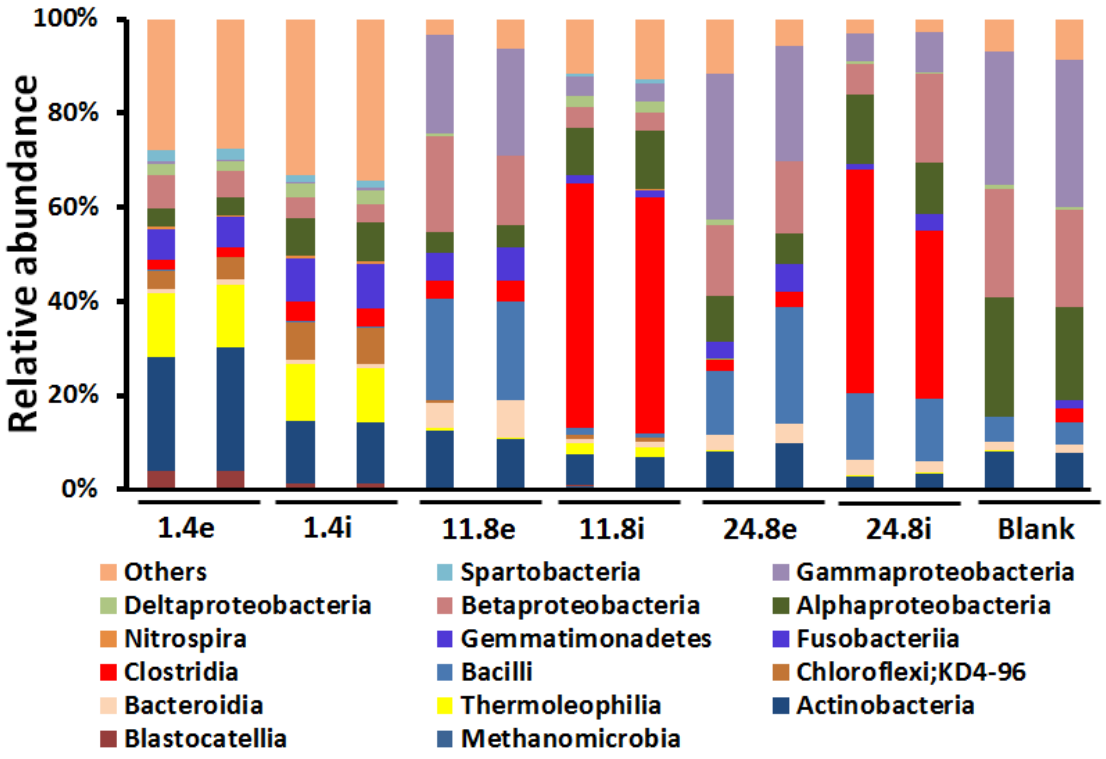

Figure S7 Comparison of microbial community at class level determined by 16S rRNA amplicon sequencing.

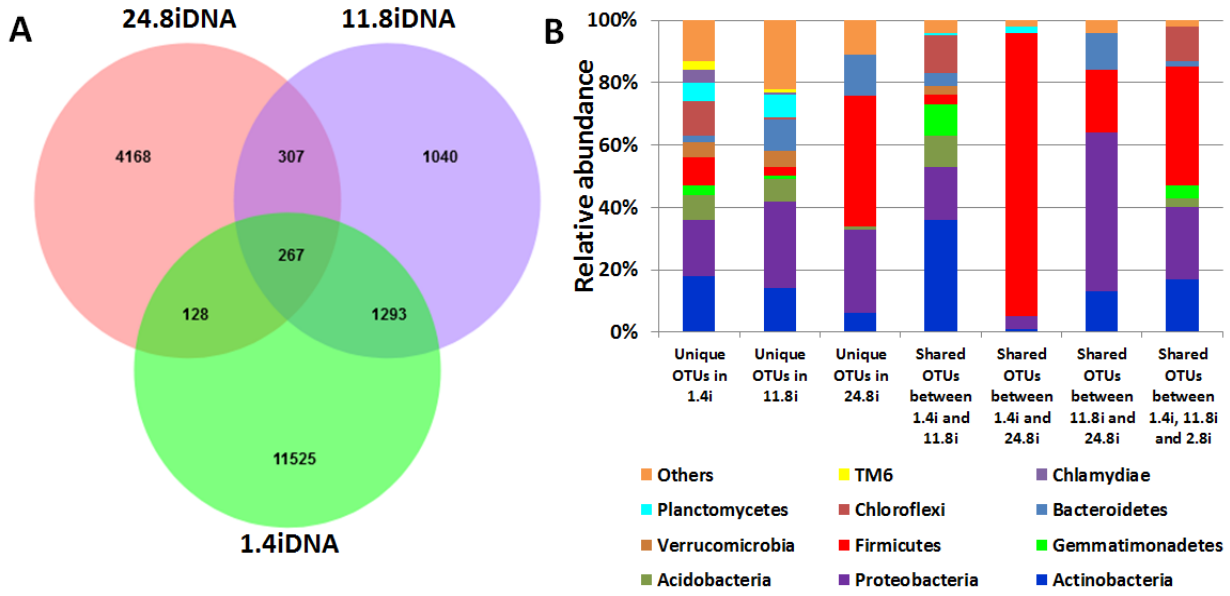

Figure S8 Venn diagram (A) of shared and e OTUs and taxonomic distribution (B) among iDNA fractions from ancient permafrost sediment at 1.4, 11.8 and 24.8 m.

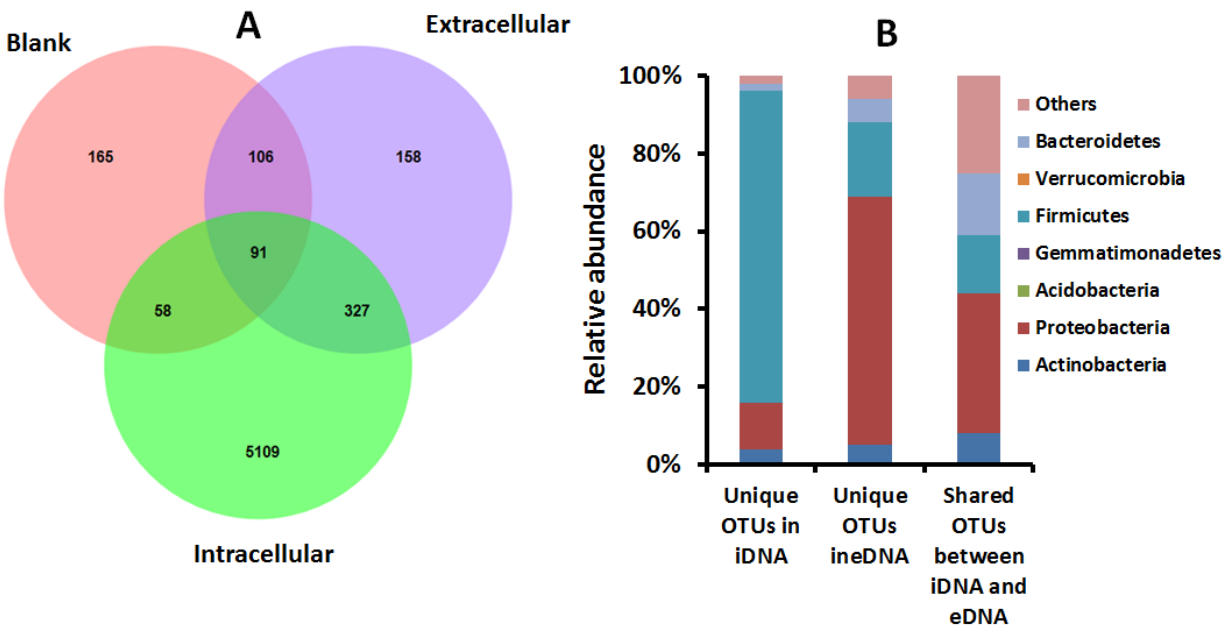

Figure S9 Venn diagram (A) of shared OTUs and taxonomic distribution (B) between iDNA and eDNA fractions from the oldest sediment at 24.8 m.